

Amendment to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

Claims 1-47 (previously cancelled)

48. (Currently amended) A method of generating a ~~hybrid~~ pluripotent mammalian cell comprising:
- (a) preparing ~~a more than one~~ cytoplasmic fragment from a mammalian oocyte or fertilized zygote ~~(the cytoplasmic donor)~~;
 - (b) obtaining ~~preparing a cell with a donor nucleus or a karyoplast with a donor nucleus (nuclear donor) which is a cell or karyoplast taken from any mammalian species a mammal~~; and
 - (c) ~~fusing~~ combining ~~said a~~ cytoplasmic fragment of step a) with the nuclear donor said cell or ~~said~~ karyoplast of step b) to produce a pluripotent mammalian cell ;
~~thereby producing a hybrid mammalian cell.~~
49. (Currently Amended) The method of claim 48, wherein ~~said the~~ cytoplasmic fragment is produced by vortexing ~~said the~~ mammalian oocyte or fertilized zygote.
50. (Currently Amended) The method of claim 48, wherein the mammalian oocyte or fertilized zygote is surrounded by a zona pellucida and wherein the zona pellucida of said mammalian oocyte or fertilized zygote is removed prior to step a).
51. (Currently Amended) The method of claim 50, wherein ~~said the~~ zona pellucida is removed by a method selected from the group consisting of: (a) treatment with an enzyme or an acidified Tyrodes solution, (b) micromanipulation followed by treatment

with a micro filament inhibitor and vortexing, ~~or~~and (c) micropipeting in the presence of an microfilament inhibitor with mechanical aspiration of cytoplasm.

52. (Currently Amended) The method of claim 51, wherein ~~said~~the enzyme is Pronase.
53. (Currently Amended) The method of claim 51, wherein ~~said~~the microfilament inhibitor is cytochalasin B.
54. (Currently Amended) The method of claim 48, wherein ~~said~~the mammalian oocyte ~~or~~ fertilized zygote, or resulting fragment thereof is enucleated.
55. (Currently Amended) The method of claim 54, wherein ~~said~~the mammalian oocyte ~~or~~ fertilized zygote, or resulting fragment thereof is enucleated by micromanipulation or centrifugation in an appropriate gradient in the presence of a microfilament inhibitor.
56. (Currently Amended) The method of claim 48, wherein ~~said~~the mammalian oocyte is matured *in vivo*.
57. (Currently Amended) The method of claim 48, wherein ~~said~~the mammalian oocyte is matured *in vitro*.
58. (Currently Amended) The method of claim 48, wherein ~~said~~the mammalian oocyte is selected from the group consisting of: an activated, low MPF maturation promotion factor ("MPF") oocyte; an aged, unactivated, low MPF oocyte; and an unactivated, high MPF, metaphase II oocyte.
59. (Currently Amended) The method of claim 58, wherein ~~said~~the mammalian oocyte is an unactivated, high MPF, metaphase II oocyte.
60. (Currently Amended) The method of claim 48, wherein ~~said~~the cytoplasm ~~donor~~fragment is from a different species from that of the nuclear donor.
61. (Currently Amended) The method of claim 48, wherein ~~said~~the cytoplasm ~~donor~~fragment is from the same species as that of the nuclear donor.

62. (Currently Amended) The method of claim 48, wherein ~~said~~the cytoplasm donor is derived ~~from~~ fragment is prepared from a mammalian oocyte or fertilized zygote taken from a non-human mammalian species.
63. (Currently Amended) The method of claim 62, wherein ~~said~~the cytoplasm donor is derived ~~from~~ fragment is prepared from a mammalian oocyte or fertilized zygote taken from a mouse, rat, rabbit, sheep, goat, pig, or cow.
64. (Currently Amended) The method of claim 63, wherein ~~said~~the cytoplasm donor is derived ~~from~~ fragment is prepared from a mammalian oocyte or fertilized zygote taken from a cow.
65. (Currently Amended) The method of claim 48, wherein ~~said~~the nuclear donor cell is ~~derived from~~ selected from the group consisting of fibroblasts, skin fibroblasts, leukocytes, granulosa cells, cumulus cells, oviductal epithelium, mammary gland cells, fetal fibroblasts, keratinocytes, hepatocytes, respiratory epithelial cells, neuronal cells, CD34[43]+ stem cells, granulocytes, or and mononuclear peripheral blood cells.
66. (Currently Amended) The method of claim 48, wherein ~~said~~the nuclear donor cell is a karyoplast.
67. (Currently Amended) The method of claim 66, wherein ~~said~~the karyoplast is an interphase cell.
68. (Currently Amended) The method of claim 48, further comprising maintaining the pluripotency by placing the cell in a culture media that supports development and proliferation while maintaining the dedifferentiated state ~~wherein said karyoplast is cytoplasm deficient.~~
69. (Withdrawn, currently amended) The method of claim 66, wherein ~~said~~the karyoplast is enriched with ~~mitochondria~~ mitochondria.

70. (Currently Amended) The method of claim 48, wherein ~~said the fusing~~combining of ~~said the~~ cytoplasmic fragment with ~~said the~~ nuclear donor is mediated by electrical fusion, chemical fusion, viruses, liposomes or cell surface proteins.
71. (Currently Amended) The method of claim 70, wherein ~~said the fusing~~combining is mediated by electrical fusion.
72. (Currently Amended) The method of claim 70, wherein ~~said the fusing~~combining is mediated by polyethylene glycol or high pH-low osmolarity.
73. (Previously Presented) The method of claim 48, further comprising an activation step.
74. (Currently Amended) The method of claim 73, wherein said activation occurs before ~~said the fusing~~combining step.
75. (Currently Amended) The method of claim 73, wherein ~~said the~~ the activation occurs after ~~said the fusing~~combining step.
76. (Currently Amended) The method of claim 73, wherein ~~said the~~ the activation is mediated by electrical pulse, ~~ionomycin~~ ionomycin/DMAP, cytochalasin/cyclohexamide, strontium, adenophostin, disintegrin RGD peptide, DDT/thimerosal, ethanol or sperm factor.
77. (Currently Amended) The method of claim 48, wherein ~~said the donor nucleus~~nuclear donor is from an embryonic, fetal, or adult cell/~~karyoplast~~, or an embryonic, fetal, or adult karyoplast.
78. (Currently Amended) The method of claim 48, wherein ~~said the donor nucleus~~nuclear donor is a diploid cell or is taken from a diploid cell.
79. (Currently Amended) The method of claim 78, wherein ~~said the donor nucleus~~nuclear donor is from a cell or karyoplast nonsynchronized, synchronized in G0/G1; or by a cell or karyoplast arrested at the G1/S border.

80. (Currently Amended) The method of claim 48, wherein ~~said the donor nucleus~~nuclear donor is optionally matched to the cell cycle stage of the cytoplasm donor.
81. (Currently Amended) The method of claim 48, wherein ~~said the donor nucleus~~nuclear donor is from a ~~differentiated or undifferentiated~~ stem cell, or differentiated or undifferentiated somatic cell.
82. (Currently Amended) The method of claim 48, wherein ~~said the donor nucleus~~nuclear donor is from a human, cow, bull, pig, sheep, goat, ~~camel, water buffalo,~~ primate, rodent or lagomorph.
83. (Currently Amended) The method of claim 48, wherein ~~said the donor nucleus~~nuclear donor is from a human.
84. (Currently Amended) The method of claim 48, wherein the ~~donor nucleus~~nuclear donor has been genetically modified.
85. (Currently Amended) The method of claim 84, wherein ~~said the donor nucleus~~nuclear donor is genetically modified with a gene designed to correct a genetic defect or ~~supply~~ cells with a capacity to produce a protein, enzyme, enzyme product, cellular component or a therapeutic agent.
86. (Withdrawn; Currently Amended) The method of claim 48, wherein the mitochondria of the donor cytoplasm is made replication incompetent.
87. (Withdrawn; Currently Amended) The method of claim 86, wherein ~~said the donor cytoplasm or~~ cytoplasm fragment is incubated with an inhibitor of mitochondrial DNA replication.
88. (Withdrawn; Currently Amended) The method of claim 86, wherein ~~said the donor cytoplasm or~~ cytoplasm fragment is incubated with an inhibitor of mitochondrial DNA replication.

89. (Withdrawn; Currently Amended) The method of claim 86, wherein ~~said~~the ~~donor cytoplasm or cytoplasm~~ fragment is incubated with EtBr.
90. (Withdrawn; Currently Amended) The method of claim 48, wherein the ~~hybrid~~ cell is supplemented with mitochondria derived from the same species as the nuclear donor.
91. (Withdrawn) The method of claim 90, wherein mitochondria is derived from the same animal or individual as the nuclear donor.
92. (Withdrawn; Currently Amended) The method of claim 90, wherein ~~said~~the mitochondria supplementation is mediated by fusion of an enucleated cytoplasm with the ~~hybrid~~ cell.
93. (Withdrawn; Currently Amended) The method of claim 92, wherein ~~said~~the enucleated cytoplasm is derived from platelets.
94. (Withdrawn; Currently Amended) The method of claim 48, wherein ~~said~~the nuclear donor ~~cell~~ is stably transfected with a gene encoding a mitochondrial maintenance factor.
95. (Withdrawn; Currently Amended) The method of claim 94, wherein ~~said~~the gene is mtTFA.
96. (Withdrawn; Currently Amended) The method of claim 48, wherein ~~said~~the nuclear donor ~~cell~~ is transiently transfected with a gene encoding a modulator of histone acetylation or a modulator of chromatin structure.
97. (Withdrawn; Currently Amended) The method of claim 96, wherein ~~said~~the gene is histone deacetylase.
98. (Currently Amended) The method of claim 48, further comprising the step of establishing a population of ~~hybrid~~ pluripotent cells derived from ~~said~~the ~~hybrid~~ pluripotent cell.
99. (Currently Amended) A ~~hybrid~~ pluripotent cell generated by the method of claim 48.
100. (Currently Amended) A population of ~~hybrid~~ pluripotent cells generated by the method of claim 98.

101. (Withdrawn; Currently Amended) The method of claim 98, further comprising the step of culturing the ~~hybrid~~ pluripotent cell population in the presence of compounds or factors which induce gene transcription, thereby producing an activated ~~hybrid~~ pluripotent cell population.
102. (Withdrawn; Currently Amended) The method of claim 101, wherein ~~said~~the compounds or factors is a reversible inhibitor of histone deacetylase.
103. (Withdrawn; Currently Amended) The method of claim 102, wherein ~~said~~the reversible inhibitor of histone deacetylase is butyrate.
104. (Withdrawn; Currently Amended) The method of claim 102, wherein ~~said~~the reversible inhibitor of histone deacetylase is trichostatin A.
105. (Withdrawn; Currently Amended) The method of claim 101, further comprising the step of culturing the activated ~~hybrid~~ pluripotent cell population in a medium that maintains the dedifferentiated state of the activated ~~hybrid~~ pluripotent cell population and support the development and proliferation of the activated ~~hybrid~~ pluripotent cell population.
106. (Withdrawn; Currently Amended) The method of claim 105, wherein ~~said~~the medium comprises cytokines, L , steel factor, or CGT44.
107. (Withdrawn; Currently Amended) The method of claim 105, wherein ~~said~~the medium comprises a feeder layer of mitotically inactivated primary fibroblast cells.
108. (Withdrawn; Currently Amended) The method of claim 105, further comprising the step of removing activated ~~hybrid~~ pluripotent cell population from ~~said~~the medium and culturing ~~said~~the activated ~~hybrid~~ pluripotent cell population in a second medium which induces differentiation of embryonic stem cells.
109. (Withdrawn; Currently Amended) The method of claim 108, wherein ~~said~~the second medium comprises a factor which induces neural pathway differentiation.

110. (Withdrawn; Currently Amended) The method of claim 109, wherein ~~said~~the factor is retinoic acid, fibroblast growth factor 2(FGF2), epidermal growth factor (EGF), or platelet-derived growth factor (PDGF).
111. (Withdrawn; Currently Amended) The method of claim 108, wherein ~~said~~the second medium comprises c-kit and erythropoietin.
112. (Withdrawn; Currently Amended) The method of claim 108, wherein ~~said~~the second medium comprises macrophage colony stimulating factor (M-CSF), inter[~~e~~]leukin I and interleukin 3.
113. (Withdrawn; Currently Amended) The method of claim 108, wherein ~~said~~the second medium comprises retinoic acid, insulin, and tri-iodothyronine.
114. (Withdrawn; Currently Amended) The method of claim 108, wherein ~~said~~the second medium comprises retinoic acid and dibutyl cyclic AMP.
115. (Withdrawn; Currently Amended) The method of claim 108, wherein ~~said~~the second medium comprises cells from the pancreatic bud.
116. (Withdrawn; Currently Amended) The method of claim 98, further comprising the step of transfecting cells of the ~~hybrid~~ pluripotent cell population with genes encoding activators or transcription factors.
117. (Withdrawn; Currently Amended) The method of claim 98, wherein ~~said~~the cells are transfected with Myo D, PPAR gamma, or C/EBP alpha.
118. (Withdrawn; Currently Amended) A method of generating and enriching a population of ~~hybrid~~ pluripotent cells comprising:
- (a) preparing a population of cytoplasts fragments stained with a first color;
 - (b) preparing a population of nuclear donor cells transfected with a gene that encodes a fluorescent protein, which is capable of fluorescing a second color;

- (c) fusing said population of cytoplasm fragments and said population of nuclear donor cells, thereby producing a population of products comprising fused products, unfused cytoplasm fragments, and unfused nuclear donors, wherein ~~said~~the fused products comprise ~~hybrid~~ pluripotent cells with a normal karyotype and aneuploidy cells;
 - (d) sorting the population of products by selecting for fused products and unfused cytoplasm marked by the first color; and
 - (e) further sorting the fused products by selecting for cells with a normal karyotype and marked by the second color.
119. (Withdrawn; Currently Amended) A method of generating and enriching a population of ~~hybrid~~ pluripotent cells comprising:
- (a) preparing a population of stained cytoplasm stained with a first color;
 - (b) preparing a population of nuclear donor cells, wherein the DNA of ~~said~~the nuclear donor cell is stained with a second color;
 - (c) fusing said population of cytoplasm and said population of nuclear donor cells, thereby producing a population of products comprising fused products, unfused cytoplasm, and unfused nuclear donors, wherein said fused products comprise ~~hybrid~~ pluripotent cells with a normal karyotype and aneuploidy cells;
 - (d) sorting the population of products by selecting for fused products and unfused cytoplasm marked by the first color; and
 - (e) further sorting the fused products by selecting for cells with a normal karyotype marked by the second color.
120. (New) The method of claim 48 wherein more than 10 cytoplasm fragments are prepared.
121. (New) The method of claim 48 wherein 10 to 50 cytoplasm fragments are prepared.

122. (New) The method of claim 48 wherein the pluripotent mammalian cell is not totipotent.
123. (New) A method for reprogramming mammalian cells comprising:
- (a) preparing more than one cytoplasmic fragment from a mammalian oocyte or fertilized zygote;
 - (b) obtaining a nuclear donor cell or karyoplast taken from a mammal; and
 - (c) combining a cytoplasmic fragment of step a) with the nuclear donor cell or karyoplast of step b) to produce a reprogrammed mammalian cell.
124. (New) A method for reprogramming mammalian cells comprising:
- (a) preparing more than one cytoplasmic fragment from a mammalian oocyte or fertilized zygote;
 - (b) obtaining a nuclear donor cell or karyoplast taken from a mammal; and
 - (c) combining cytoplasmic fragments of step a) with the nuclear donor cell or karyoplast of step b) to produce a reprogrammed mammalian cell.
125. (New) The method of claim 123 or 124 wherein the reprogramming is facilitated by the use of chemical or biologically derived agents known to cause gene reactivation.
126. (New) The method of claim 123 or 124, wherein the reprogrammed mammalian cell is a cardiomyocyte.
127. (New) A method of generating a hybrid pluripotent mammalian cell comprising:
- (a) preparing more than one cytoplasmic fragment from a mammalian oocyte or fertilized zygote;
 - (b) obtaining nuclear donor cell or karyoplast taken from a mammal; and
 - (c) combining cytoplasmic fragments of step a) with the nuclear donor cell or karyoplast of step b) to produce a pluripotent mammalian cell.